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Pathogenesis of Skin Lesions in Mice with Chronic Proliferative Dermatitis (*cpdm/cpdm*)

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Chronic proliferative dermatitis is a spontaneous mutation in C57BL/Ka mice (cpdm/cpdm), showing alopecia, epithelial hyperproliferation, infiltration by eosinophils and macrophages, and vascular dilatation. To further elucidate its pathogenesis, organs of 1-, 2-, 3-, 4-, 5-, and 6-week-old cpdm/cpdm mice were examined. At 4 weeks, the epidermal thickness was increased, whereas already at 3 weeks, the bromodeoxyuridine incorporation was increased in the basal keratinocytes. However, already at the age of 1 week, skin, lungs, and lymph nodes were infiltrated by eosinophils although no macroscopic lesions were present. Compared with control animals, 6-week-old cpdm/cpdm mice had decreased serum IgE levels and increased numbers of mast cells. From the age of 1 week these mast cells became increasingly IgE positive. In contrast, the mast cells of the control animals remained IgE negative. Mast cells of control and cpdm/cpdm mice were interleukin-4 and tumor necrosis factor-α positive. A likely explanation for the tissue infiltration of eosinophils could be the release of interleukin-4 and tumor necrosis factor-α from activated mast cells. Tumor necrosis factor-α may lead to the expression of E-selectin on endothelial cells, facilitating interleukin-4-mediated eosinophil transendothelial migration. Although various pathogenetic aspects of the cpdm/cpdm mouse need further elucidation, this model can be a tool to study eosinophil infiltration, leukocyte-endothelial cell interactions, and mast cell proliferation. Furthermore, the cpdm/cpdm mouse can be used to study chronic inflammatory skin disease because of the severe epidermal proliferation. (Am J Pathol 1996, 148:941–950)

The detection and study of hereditary disorders in animals have greatly contributed to our understanding of the complex mechanisms underlying disease processes. We recently described a mouse mutant (*cpdm/cpdm*) with chronic proliferative dermatitis on a C57BL/Ka background.[1–4] The skin lesions are characterized by erythema, severe nonscarring hair loss, and scaling at the age of 5 weeks. Light microscopically, the lesions are hyper- and parakeratosis, hyperplasia and apoptosis of keratinocytes, vascular proliferation, and infiltration of mast cells, macrophages, and granulocytes, mainly eosinophils. Only few T cells are present in the skin. Similar lesions as found in the skin are observed in the esophagus and forestomach. In addition, there are mixed cellular infiltrates in the liver, lungs, and perisynovial connective tissue. Both the draining lymph nodes of the skin and the spleen are enlarged due to heavy infiltration of eosinophils, whereas marked extramedullary myelopoiesis accounts for the enlargement of the spleen. The lesions regressed upon systemic treatment with corticosteroids.[1]

Transfer of hemopoietic cells from *cpdm/cpdm* mice to lethally irradiated syngeneic mice failed to induce *cpdm/cpdm* lesions in the recipients, suggesting that hemopoietic cells do not play a primary role in the pathogenesis.[1] Full-thickness grafts of affected skin from *cpdm/cpdm* mice and normal skin from C57BL/Ka mice transplanted onto *cpdm/cpdm*, C57BL/Ka mice or athymic nude mice maintain the donor phenotypes.[3] These findings suggest that the

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pathological features of the *cpdm/cpdm* mice are the result of a disorder in the dermis or epidermis and not caused by a systemic defect.

To elucidate the pathogenesis of the chronic skin disease in the *cpdm/cpdm* mouse, additional insight in the development of the lesions is necessary. Therefore, the skin and other affected organs of 1-, 2-, 3-, 4-, 5-, and 6-week-old *cpdm/cpdm* mice and appropriate controls were examined. Here, we report which histological changes occur in the various organs and how these changes relate to macroscopic observable skin lesions.

Materials and Methods

Animals

The cpd mutation (*cpdm/cpdm*) arose spontaneously in the colony of the inbred C57BL/KaLawRij mice in the specific-pathogen-free breeding facility of TNO in Rijswijk, The Netherlands. The microbiological status is checked regularly by routine serological, bacteriological, and histological procedures. Two breeding models were used. 1) Females, heterozygous for the cpd mutation (+/*cpdm*) were bred with homozygous males (*cpdm/cpdm*). These males cannot be older than 11 weeks because the fertilizing capacity decreases. This was not due to atrophy of the reproductive organs but to an overall weakened systemic condition resulting from severe pruritus. 2) Heterozygous females (+/*cpdm*) were bred with heterozygous males (+/*cpdm*). The current investigation was carried out with the offspring of both breeding models. Eight mice (four *cpdm/cpdm* and four littermate controls) per week were macroscopically examined at the age of 1, 2, 3, 4, 5, and 6 weeks, weighed, killed, necropsied, and microscopically examined. Mice used for serum IgE determination were sacrificed at 8 weeks.

Histology

All tissues were fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin. Three-micron sections were routinely stained with hematoxylin and eosin (H&E). The selected tissues with *cpdm/cpdm* lesions, ie, skin, tongue, esophagus, forestomach, lung, liver, spleen, and lymph nodes, were given a score corresponding to the degree of cellular (granulocytic and monocytic) infiltration as follows: no infiltration, 0; minimal infiltration, 1; mild, 2; moderate, 3; severe, 4; and marked, 5.

Immunohistochemistry

Skin samples were quick-frozen in liquid nitrogen for immunohistochemistry. Cryostat sections of the skin of animals at the age of 1 and 6 weeks were stained using an indirect peroxidase method. Sections were fixed in acetone, washed in phosphate-buffered saline, and incubated with anti E-selectin (10E6[5]; kindly provided by Dr. B. Wolitzky), anti P-selectin (RW40.34/4[6]), anti-IgE (EM-95[7]; kindly provided by Dr. M. Baniyash), biotinylated anti-tumor-necrosis-factor (TNF)-α (61E71[8]) anti-interleukin (IL)-4 (11B11[9]), or biotinylated anti-IL-5 (TRFK4[10]) for 60 minutes at room temperature. Thereafter, sections were washed and incubated with avidin-peroxidase (Dakopatts, Copenhagen, Denmark) or rabbit anti-rat peroxidase (Dakopatts) for 60 minutes at room temperature. Peroxidase activity was visualized with diaminobenzidine (Sigma Chemical Co., St. Louis, MO). Some sections incubated with anti-IgE were counterstained with alcian blue.

Bromodeoxyuridine (BrdU) Labeling

Thirty minutes before being killed, mice were administered 0.625 mg of BrdU (Sigma) intraperitoneally to determine the rate of cell proliferation in the epidermis. Skin was fixed for 18 hours in neutral-buffered formalin, stored in 70% alcohol, and later embedded in paraffin. Paraffin-embedded sections were deparaffinized, rehydrated, and incubated with monoclonal anti-BrdU antibody (Dakopatts). The labeled nuclei of the slides incubated with anti-BrdU were visualized by peroxidase-labeled rabbit anti-mouse Ig (Dakopatts), followed by diaminobenzidine in combination with 1% cobalt chloride to enhance the staining intensity.

Total Serum IgE Determination

Total serum IgE levels were measured in nine control C57BL/Ka and three *cpdm/cpdm* mice of 8 weeks old by isotype-specific enzyme-linked immunosorbent assay as described previously.[11] Plates were coated with monoclonal RaAM/IgE (EM95; 2μg/ml) and incubated overnight at 4°C with diluted serum samples. Detection was based upon addition of biotinylated RaAM/IgE (2 μg/ml; clone R35–118, Pharmagen, San Diego, CA) and, after washing, the conjugate streptavidin-peroxidase (Jackson Immunoresearch Laboratories, West Grove, PA). The ELISA was developed by using 2,2′-amino-bis(3-ethylbenz-thioazoline-6-sulfonic acid (Sigma). The detection limit of this enzyme-linked immunosorbent assay was 0.5 ng/ml.

Morphometry and Statistical Analysis

BrdU-labeled nuclei were counted per centimeter of basement membrane. The thickness of the combined nucleated epithelial layers of the esophagus and interfollicular epidermis was measured at 10 sites. From these measurements, the mean thickness was calculated for each epithelium. The density of dermal mast cells was determined for the 1-, 3-, and 6-week-old animals in toluidine blue sections as number of cells per square millimeter of dermis. The area of the dermis was determined by subtracting the area occupied by pilosebaceous units and blood vessels from the total area of the dermis. The measurements were performed with computer-aided morphometry (Kontron-Videoplan, Zeiss, Germany). Data are presented as mean ± SEM. Statistical analysis was performed by Student's *t*-test.

Results

Breeding Efficacy

Two breeding models were used in this study. Breeding model 1 (+/*cpdm* female × +/*cpdm* male) resulted in 26% *cpdm/cpdm* descendants, with equal numbers of males and females. Breeding model 2 (+/*cpdm* female × *cpdm/cpdm* male) resulted in 45% *cpdm/cpdm* descendants, 58% males and 42% females. These results are consistent with an autosomal mode of inheritance and indicate that breeding method 2 results in the highest breeding efficiency (during the relatively short period of male mating activity).

Clinical Symptoms

The first *cpdm/cpdm* symptom consisted of thickening of the eyelids. This was observed not earlier than week 2. Three out of four animals demonstrated this symptom and could also microscopically be identified as *cpdm/cpdm* mice. At the age of 3 weeks the *cpdm/cpdm* mice had a thinner fur than control mice, and reddening of the axilla was observed in such a way that all *cpdm/cpdm* mice could be recognized. At the age of 4 weeks the dorsal neck and ventral chest developed hair loss and mild scaling and this subsequently became more severe. After 5 weeks, the animals suffered from severe pruritus demonstrated by severe scratching.

From the age of 5 weeks, the *cpdm/cpdm* animals showed 7% weight reduction compared with the control animals. At the age of 6 weeks this increased to 12% weight reduction.

Pathology

No lesions were observed in the control animals. Gross examination revealed skin changes in the *cpdm/cpdm* mice as described above. Furthermore, at the age of 3 weeks the deep and superficial cervical, thoracic, axillary, and brachial lymph nodes and the spleen were mildly enlarged. Detailed examination of the skin, lymph nodes, lung, tongue, esophagus, forestomach, liver, and spleen led to the observations described below. In other organs, no macroscopic or microscopic abnormalities were observed. Special stains for the detection of yeasts, fungi, and parasites were consistently negative. Also, serological, bacteriological, and histological measurements for the microbiological status were consistently negative. The results of semiquantitative measurements of cellular infiltration at different locations are represented in Figure 1, a–h.

Skin

At the age of 1 week, the dermis of the *cpdm/cpdm* mice was already mildly infiltrated with inflammatory cells, predominantly eosinophils and some monocytes (Figure 2a). This picture was similar at the age of 2 weeks. At 3 weeks, a folliculitis with some degenerated hairshafts, some apoptosis of the keratinocytes, dilatation of the capillaries, and a moderate infiltration of inflammatory cells in the dermis were present. This became more severe at 4 weeks, at which age also acanthosis (Figure 2b), multifocal parakeratosis, and hyperkeratosis had developed. The lesions were more advanced in 5-week-old mice with intracorneal microabscesses present in two animals. At 6 weeks, the lesions were quite extensive and severe.

Lymph Nodes

At the age of 1 week, the deep and superficial cervical, thoracic, axillary, and brachial lymph nodes showed acute lymphadenitis and perilymphadenitis mainly characterized by eosinophil infiltration in the cortex and medulla sometimes obscuring the normal architecture (Figure 3). The lymphadenitis varied from mild to severe, but the severity did not change with age.

Lungs

Already at the age of 1 week lesions in the lungs were observed. Predominantly perivascularly, but in two animals also interstitially, mild cellular infiltration

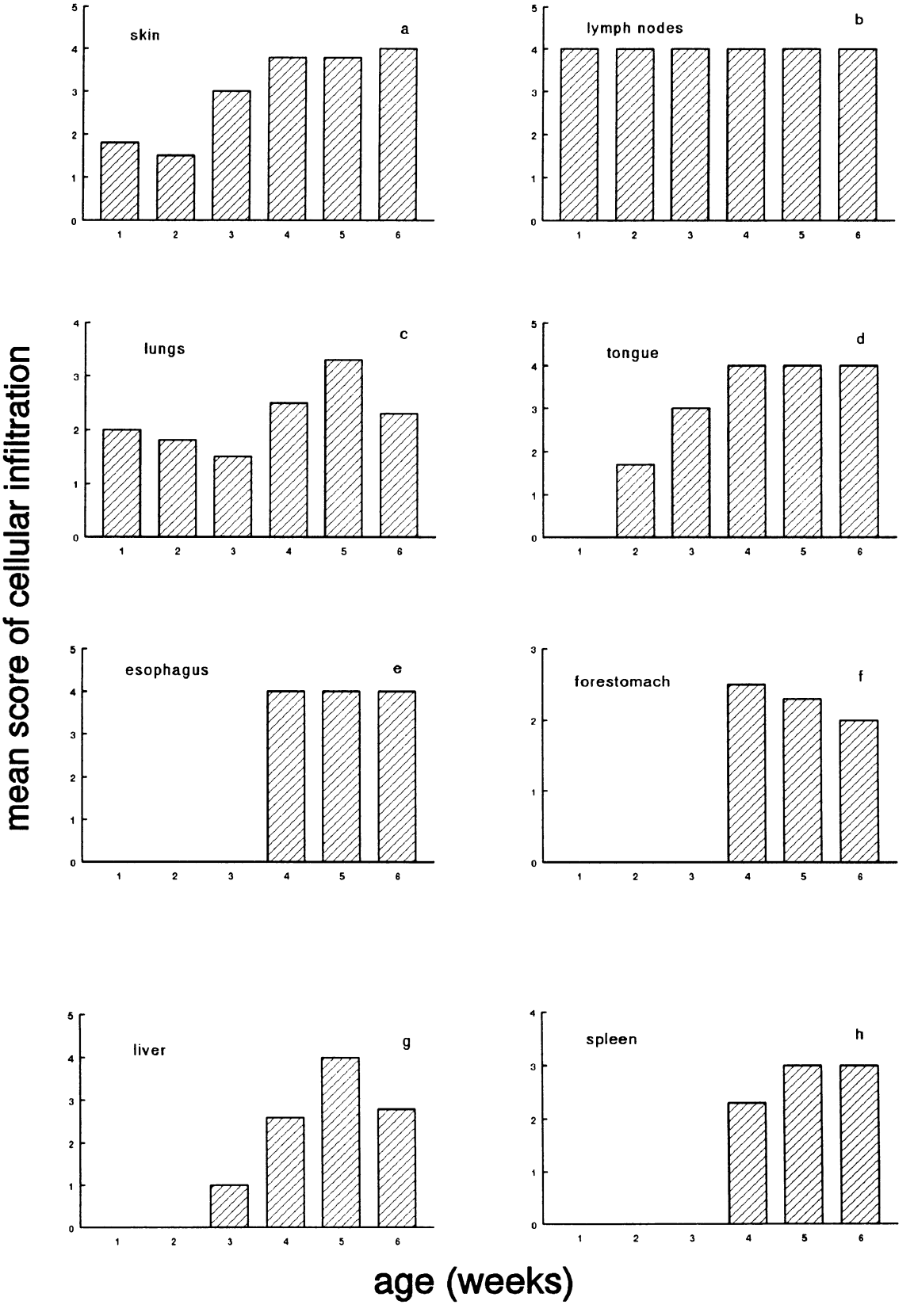

Figure 1. *Mean scores (n = 4) of cellular infiltration (monocytes and granulocytes), in excess of (negligible) values obtained in controls, in the skin* (a), *deep and superficial cervical, thoracic, axillary, and brachial lymph nodes* (b), *lungs* (c), *tongue* (d), *esophagus* (e), *forestomach* (f), *liver* (g), *and spleen* (h) *of* cpdm/cpdm *mice at the age of 1 to 6 weeks.*

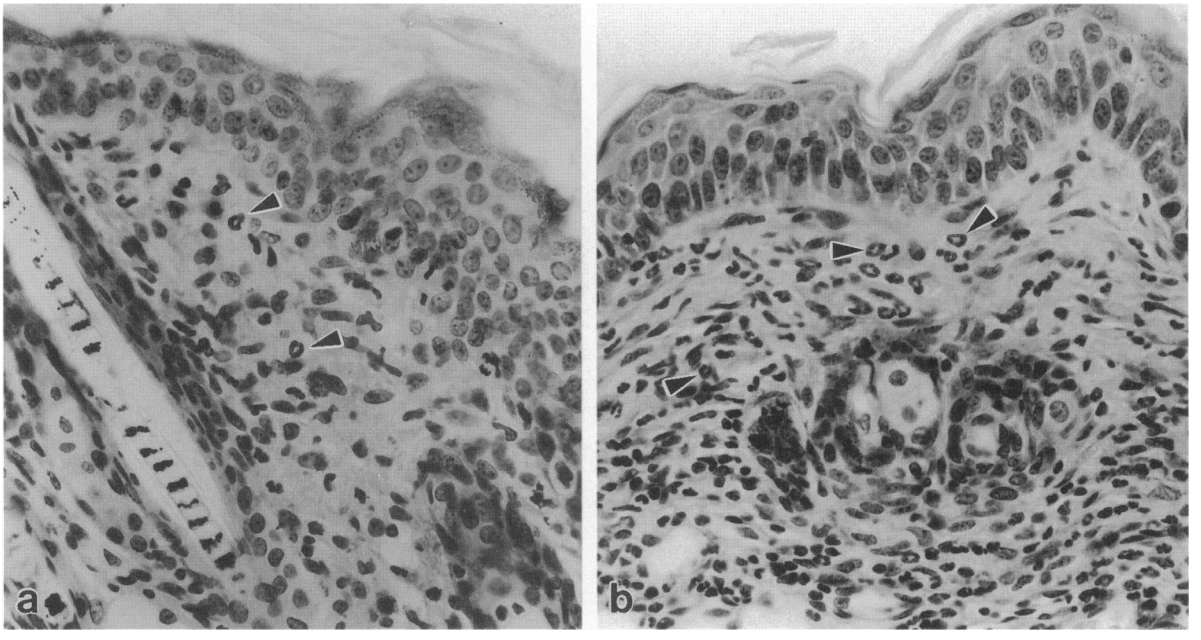

Figure 2. *Skin of a 1-week-old* (**a**) *and a 4-week-old* (**b**) cpdm/cpdm *mouse. At the age of 1 week, the dermis of the* cpdm/cpdm *mouse is mildly infiltrated with predominantly eosinophils* (**a, arrowheads**), *whereas at 4 weeks a severe cellular infiltration is observed* (**arrowhead**) *and acanthosis has developed* (**b**). *H&E; magnification, ×380.*

was present. These inflammatory cells were predominantly eosinophils with some monocytes. At the age of 2 and 3 weeks the lesions had not changed appreciably. However, at the age of 4 weeks, the four *cpdm/cpdm* mice had developed a mild to moderate diffuse granulomatous pneumonia. This became worse thereafter.

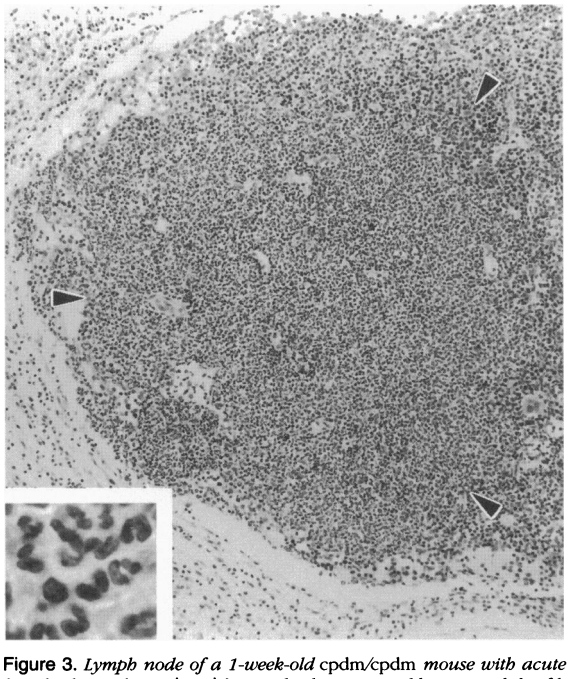

Figure 3. *Lymph node of a 1-week-old* cpdm/cpdm *mouse with acute lymphadenitis* (**arrowheads**) *mainly characterized by eosinophil infiltration* (**inset**). *H&E; magnification, ×90.* **Inset:** *H&E; magnification, ×900.*

Proximal Intestinal Tract

The tongue, esophagus, and forestomach of the mouse are lined by orthokeratinizing squamous epithelium. Although similar changes were observed as those in the skin, the time of occurrence was different. The first lesions were observed at the age of 2 weeks in the tongue, whereas lesions in the esophagus and forestomach were absent at week 3 (Figure 4a) and appeared at the age of 4 weeks (Figure 4b). The lesions were comparable to the lesions observed in the skin. There were two exceptions: infiltrating cells in the epithelium of the proximal digestive tract forming intraepithelial and intracorneal spongiform pustules, and no parakeratosis was observed. These infiltrating cells were mainly eosinophils. Once the lesions had developed in the tongue and the esophagus, they were immediately moderate to severe. The lesions in the forestomach were not as pronounced as in the esophagus, and they were most severe at the junction of the forestomach and glandular stomach.

Liver

Normal extramedullary myelopoiesis was observed in the liver of 1-week-old control and *cpdm/*

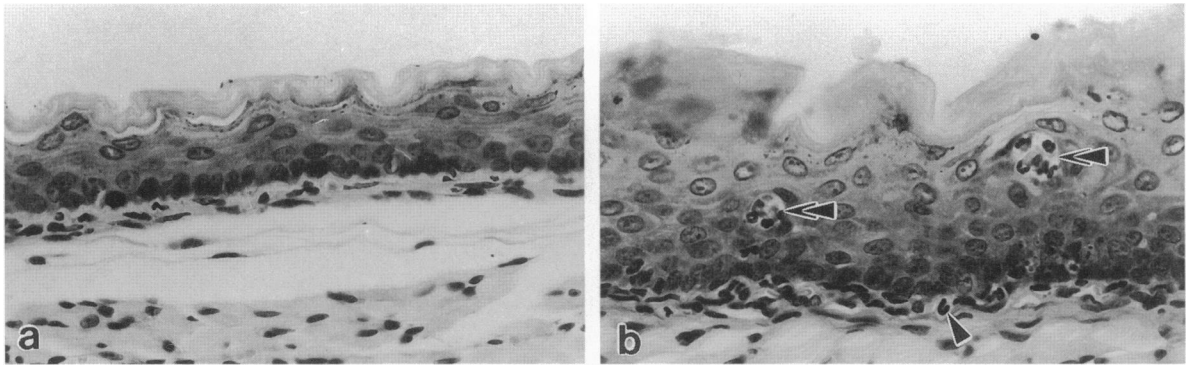

Figure 4. *Esophagus of a 3-week-old* (a) *and a 4-week-old* (b) cpdm/cpdm *mouse. No lesions are observed at 3 weeks, whereas at 4 weeks hyperkeratosis, acanthosis, intraepithelial spongiform pustules* (double arrowheads), *and subepithelial cellular infiltration* (arrowheads) *are present. H&E; magnification, ×380.*

cpdm animals. However, at the age of 2 weeks, only mild myelopoiesis was observed in the control mice, whereas the *cpdm/cpdm* mice had developed moderate myelopoiesis. At 3 weeks of age, the *cpdm/cpdm* mice showed small perivascular infiltrates consisting of eosinophils and macrophages. These lesions subsequently became more severe.

Spleen

At the age of 1 and 2 weeks, control and *cpdm/cpdm* mice both had extramedullary hematopoiesis in the spleen, but at 3 weeks of age only the *cpdm/cpdm* mice showed moderate myelopoiesis. This became more severe with age, and severe eosinophil infiltration was observed in the red and white pulp starting at the age of 4 weeks. Together with this infiltration, atrophy of the white pulp occurred.

Epithelial Thickness and Proliferation

The epidermal thickness of the skin of the *cpdm/cpdm* mice was significantly increased ($P < 0.025$) compared with the control mice at 4 weeks of age (Figure 5a) while the BrdU incorporation was significantly increased ($P < 0.025$) in the basal keratinocytes of the *cpdm/cpdm* mice already at 3 weeks (Figure 5c). The epithelial thickness (Figure 5b) and BrdU incorporation (Figure 5d) in the basal keratinocytes of the esophagus were significantly increased ($P < 0.025$ and $P < 0.05$, respectively) at 4 weeks of age.

Mast Cell Count, Serum IgE Level, and Immunohistochemistry

At the age of 1 and 3 weeks the normal (68 ± 7 and 123 ± 23 cells/mm^2, respectively) and *cpdm/cpdm* skin (75 ± 10 and 85 ± 9 cells/mm^2, respectively) had the same number of mast cells, whereas 6-week-old *cpdm/cpdm* mice had a significantly increased ($P < 0.025$) number of mast cells compared with 6-week-old control mice (213 ± 22 and 82 ± 7 cells/mm^2, respectively).

Total serum IgE levels of the *cpdm/cpdm* mice (0.05 ± 0.03 ng/mg) were decreased approximately 10-fold compared with total IgE levels of control animals (0.66 ± 0.15 ng/ml; $P < 0.025$).

No E-selectin, P-selectin, IgE, or IL-5 expression was observed in skin of control animals (Table 1). E-selectin was expressed on endothelial cells of blood vessels in the dermis of *cpdm/cpdm* mice at the age of 1, 3, and 6 weeks, whereas no P-selectin was observed in these mice (Table 1). IgE-positive mast cells were observed in the dermis at the age of 1, 3, and 6 (Figure 6) weeks of *cpdm/cpdm* mice. The mast cells of the 1-week-old *cpdm/cpdm* mice were weakly IgE positive, whereas 3- and 6- (Figure 6) week-old animals showed strongly IgE-positive mast cells (Table 1). In mast cells of 6-week-old *cpdm/cpdm* mice, there seemed to be a negative correlation between alcian blue positivity and IgE positivity; strongly alcian-blue-positive mast cells showed less dense or even negative IgE surface staining and vice versa. The mast cells of the control and *cpdm/cpdm* mice were IL-4 and TNF-α positive (Table 1). No tissue IL-5 expression was observed (Table 1), although T lymphocytes reacted positively in frozen sections of spleen using the same dilution.

Discussion

In this paper, we describe the development of the *cpdm/cpdm* phenotype in the mutant mouse on a C57BL/Ka background. The most striking observa-

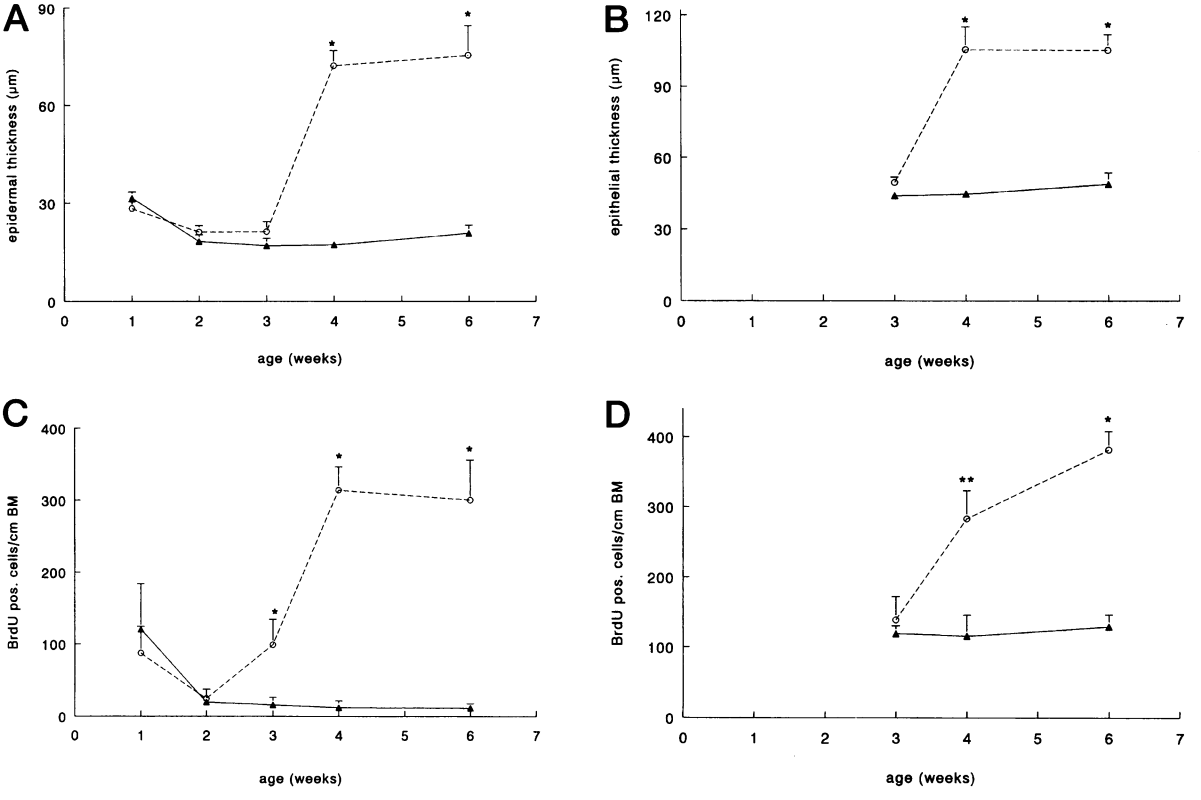

Figure 5. *Epidermal thickness* (a) *and number of BrdU-positive cells* (c) *in the skin and epithelial thickness* (b) *and number of BrdU-positive cells* (d) *in the esophagus.* ▲, *control mice;* ○, cpdm/cpdm *mice. BM, basement membrane.* Bars *indicate mean ± SEM of four mice.* *$P < 0.025$; **$P < 0.05$ (cpdm/cpdm versus *control*).

tion of this study was that, already at the age of 1 week, skin, lungs, and lymph nodes were infiltrated by eosinophils, whereas at that time point no macroscopic lesions were present. At 1 week later, similar observations were made in the tongue, and 2 weeks later, cellular infiltrations were also observed in the esophagus and forestomach. This coincided with the moment that the animals received pelleted food. At 3 weeks, the *cpdm/cpdm* lesions became macroscopically visible.

These observations leave us with the intriguing question of what mechanism leads to this selective accumulation of eosinophils. Our results seem to indicate that mast cell activation is involved in this process. Mast cell number alters and high levels of bound IgE are observed, which are not observed in control mice. An explanation for the low serum IgE levels observed in the *cpdm/cpdm* mouse could be that serum IgE is maintained at a low level due to its rapid adsorption to tissue mast cells and circulating basophils.[12,13] With the alcian blue staining, it seems that most mast cells in the *cpdm/cpdm* mice that demonstrate detectable IgE have lost their granules, especially prominent in the animals of 3 and 6 weeks old. Taken together, these observations indicate mast cell activation through IgE resulting in the loss of granule-containing mediators like histamine, proteases, and cytokines and even secretion of membrane-derived mediators like leukotrienes and prostaglandins.[14–16] However, so far no mast cell

Table 1. *Immunohistochemistry of Skin of 1-, 3-, and 6-Week-Old Control and* cpdm/cpdm *Mice*

	Control			*cpdm/cpdm*		
Age in weeks	1	3	6	1	3	6
IgE on mast cells	–	–	–	+	+++	+++
TNF-α	ND	ND	++	ND	ND	++
IL-4	+	+	+	+	+	+
IL-5	–	–	–	–	–	–
E-selectin on endothelial cells	–	–	–	++	++	++
P-selectin	–	–	–	–	–	–

ND, not done.

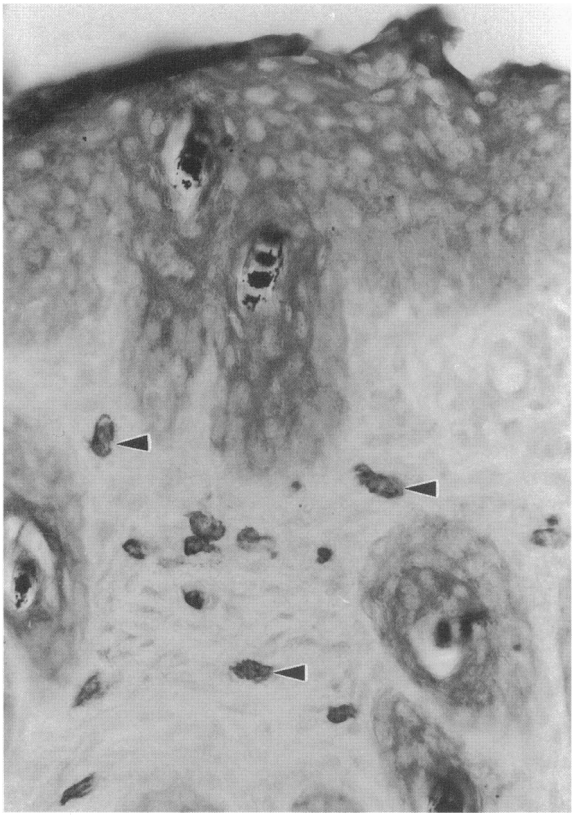

Figure 6. *IgE-positive mast cells in the dermis of a 6-week-old* cpdm/cpdm *mouse* (**arrowheads**). *Indirect immunoperoxidase with hematoxylin counterstain; magnification, ×380.*

products have been measured. It has been reported that IL-1, TNF-α, IL-3, IL-4, IL-5, granulocyte/macrophage colony-stimulating factor, IL-8, and RANTES are of importance with respect to eosinophil tissue mobilization.[17] Although we could not demonstrate IL-5 on the protein level, IL-4 and TNF-α were present in mast cells of control and *cpdm/cpdm* mice. Thus, upon IgE-mediated activation the mast cells of *cpdm/cpdm* mice may be triggered to release IL-4 or TNF-α. The release of those cytokines, but in particular that of IL-4, may provide the explanation for the selective tissue mobilization of eosinophils. Injection of IL-4 in mice recruits eosinophils selectively.[18] Furthermore, IL-4 is only chemotactic for eosinophils from the peripheral blood of patients with atopic dermatitis and not for eosinophils from normal individuals.[19,20] These arguments support the view that IL-4 could play a role in the observed eosinophil recruitment. However, as it is known that IL-4 stimulates human B cells to produce IgE[15,21] and elevated IgE levels are not observed in the *cpdm/cpdm* mouse, the possibility of another factor cannot be excluded at this moment. The involvement of cytokines that can be produced by monocytes and keratinocytes like RANTES and IL-8 have to be investigated.[22,23] TNF-α released from the mast cells can play a role in the transendothelial migration of eosinophils into the dermis. Transendothelial migration of eosinophils into the tissue involves a cumulative series of interactions in which adhesion molecules on endothelial cells play a crucial role. E-selectin expression is observed already at the age of 1 week and is still seen at the age of 6 weeks in *cpdm/cpdm* mice. On the other hand, P-selectin was not observed. E-selectin is transcriptionally induced by cytokines such as TNF-α or IL-1β.[24] Therefore, our findings concerning the observed E-selectin expression in the developing *cpdm/cpdm* mice indicate the release of TNF-α, probably from mast cells. It was hypothesized by Bosse[25] that P-selectin mediates very early adhesion events, whereas E-selectin would act later and maybe even replace P-selectin at later steps in the inflammatory process. Therefore, the age of 1 week of the *cpdm/cpdm* mice may even be too late for the detection of P-selectin, as this adhesion molecule on the endothelial cells may have been replaced by E-selectin. In a more chronic stage of the inflammatory process of the *cpdm/cpdm* mice, E-selectin could not be observed (H. I. Gallardo Torres, personal communication). It has been reported that in patients with atopic dermatitis E-selectin is a critical adhesion molecule, not only in the acute stage but also in the chronic skin lesions. In this paper, E-selectin is considered responsible for the tissue infiltration of memory T cells,[26] whereas in the *cpdm/cpdm* mouse it should be involved in eosinophil tissue infiltration. Taken together, in the early development of the *cpdm/cpdm* lesion, IgE-mediated mast cell activation leading to the release of the cytokines IL-4 and TNF-α could be responsible for the observed eosinophil tissue infiltration. In this concept IL-4 should lead to the endothelial expression of an as yet unidentified adhesion structure, allowing selective eosinophil tissue infiltration.

The earlier finding that no lesions could be observed after transfer of hemopoietic cells from spleen or bone marrow from *cpdm/cpdm* mice to syngeneic control animals suggested that cells such as mast cells do not play a primary role in its pathogenesis.[1] The maturation of mast cells is regulated by many factors including certain cytokines.[27,28] What cell type and which factors are most important for the development of the *cpdm/cpdm* mouse therefore remains a subject for additional investigation.

Microscopically as well as macroscopically, no epidermal thickening can be observed in the *cpdm/cpdm* mice at the age of 3 weeks, whereas the BrdU incorporation is already increased. At the age of 4 weeks the increased epidermal thickness becomes

obvious microscopically as well as macroscopically. A possible explanation for the proliferation of the keratinocytes could be that accumulation of recruited eosinophils leads to enhanced TGF-α concentrations and thus to epidermal proliferation and thickening.[29]

In conclusion, the *cpdm/cpdm* mouse can be a valuable tool to study eosinophil tissue infiltration, leukocyte-endothelial cell interactions, and mast cell proliferation. Furthermore, the *cpdm/cpdm* mouse can be used to study chronic inflammatory skin disease because of the severe epidermal proliferation.

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References

1. HogenEsch H, Gijbels MJJ, Offerman E, van Hooft J, van Bekkum DW, Zurcher C: A spontaneous mutation characterized by chronic proliferative dermatitis in C57BL mice. Am J Pathol 1993, 143:972–982
2. Gijbels MJJ, HogenEsch H, Blauw B, Roholl P, Zurcher C: Ultrastructure of keratinocytes of mice with chronic proliferative dermatitis (cpd). Ultrastruct Pathol 1995, 19:107–111
3. Gijbels MJJ, HogenEsch H, Bruijnzeel PLB, Elliott GR, Zurcher C: Maintenance of donor phenotype after full-thickness skin transplantation from mice with chronic proliferative dermatitis (*cpdm/cpdm*) to C57BL/Ka and nude mice and vice versa. J Invest Dermatol 1995, 105: 769–773
4. Gijbels MJJ, Bruijnzeel PLB, Toonstra J, Elliott GR, Zurcher C, HogenEsch H: Chronic proliferative dermatitis. Comp Pathol Bull 1995, 27:2–3
5. Norton CR, Rumberger JM, Burns DK, Wolitzky BA: Characterization of murine E-selectin expression *in vitro* using novel anti-mouse E-selectin monoclonal antibodies. Biochem Biophys Res Commun 1993, 195:250–258
6. Gotsch U, Jäger U, Dominis M, Vestweber D: Expression of P-selectin on endothelial cells is upregulated by LPS and TNF-α *in vivo*. Cell Adhes Commun 1994, 2:7–14
7. Baniyash M, Eshar Z: Inhibition of IgE binding to mast cells and basophils by monoclonal antibodies to murine IgE. Eur J Immunol 1984, 14:799–804
8. Leeuwenberg JFM, Van Damme J, Maeger T, Jeunhomme TMAA, Buurman WA: Effects of tumor necrosis factor on the interferon-γ-induced major histocompatibility complex class II antigen expression by human endothelial cells. Eur J Immunol 1988, 18:1469–1472
9. O'hara J, Paul WE: Production of monoclonal antibody to and molecular characterization of B-cell stimulatory factor-I. Nature 1985, 315:333–336
10. Schumacher JH, O'Garra A, Shrader B, Van Kimmenade A, Bond MW, Mossmann TR, Coffman RL: The characterization of four monoclonal antibodies specific for mouse IL-5 and development of mouse and human IL-5 enzyme-linked immunosorbent assay. J Immunol 1988, 141:1576–1581
11. Coffman RL, Carty J: A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-γ. J Immunol 1986, 136:949–954
12. Benyon RC, Church MK: Mast cells in the skin. Handbook of Atopic Eczema. Edited by T Ruzicka, J Ring, B Przybilla. Berlin, Springer Verlag, 1991, pp 173–185
13. Malveaux FJ, Conroy MC, Adkinson NF, Lichtenstein LM: IgE receptors on human basophils. J Clin Invest 1978, 62:176–181
14. Galli SJ: New concepts about the mast cell. N Engl J Med 1993, 328:257–265
15. De Vries JE: Novel fundamental approaches to intervening in IgE-mediated allergic diseases. J Invest Dermatol 1994, 102:141–144
16. Gordon JR, Burd PR, Galli SJ: Mast cells as a source of multifunctional cytokines. Immunol Today 1990, 11: 458–464
17. Bruijnzeel PLB: Eosinophil tissue mobilization in allergic disorders. Ann NY Acad Sci 1994, 725:259–267
18. Moser R, Groscurth P, Carballido JH, Bruijnzeel PLB, Blaser K, Heusser CH, Fehr J: Interleukin-4 induces tissue eosinophilia in mice: correlation with its in vitro capacity to stimulate the endothelial cell-dependent selective transmigration of human eosinophils. J Lab Clin Med 1993, 122:567–575
19. Dubois GR, Bruijnzeel-Koomen CAFM, Bruijnzeel PLB: IL-4 induces chemotaxis of blood eosinophils from atopic dermatitis patients, but not from normal individuals. J Invest Dermatol 1994, 102:843–846
20. Dubois GR, Bruijnzeel PLB: IL-4 induces migration of eosinophils in allergic inflammation. Ann NY Acad Sci 1994, 725:268–273
21. Bonnefoy J-Y, Aubry J-P, Gauchat J-F, Graber P, Life P, Flores-Romo L, Mazzei G: Receptors for IgE. Curr Opin Immunol 1993, 5:944–949
22. Kapp A, Zeck-Kapp G, Czech W, Schöpf E: The cytokine RANTES is more than a chemoattractant: characterization of its effect on human eosinophil oxidative metabolism and morphology in comparison with IL-5 and GM-CSF. J Invest Dermatol 1994, 102:906–914
23. Schröder J-M, Kameyoshi Y, Christophers E: RANTES, a novel eosinophil-chemotactic cytokine. Ann NY Acad Sci 1994, 725:91–103
24. Bevilacque MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone MA: Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Natl Acad Sci USA 1987, 84:9238–9242
25. Bosse R, Vestweber D: Only simultaneous blocking of the L- and P-selectin completely inhibits neutrophil migration into mouse peritoneum. Eur J Immunol 1994, 24:3019–3024

26. Wakita H, Sakamoto T, Tokura Y, Takigawa M: E-selectin and vascular cell adhesion of molecule-1 as critical adhesion molecules for infiltration of T lymphocytes and eosinophils in atopic dermatitis. J Cutaneous Pathol 1994, 21:33–39
27. Galli SJ: New insights into "the riddle of the mast cells": microenvironmental regulation of mast cell development and phenotypic heterogeneity. Lab Invest 1990, 62:5–33
28. Galli SJ, Wershil BK, Costa JJ, Tsai M: For better or for worse: does stem cell factor importantly regulate mast cell function in pulmonary physiology and pathology? Am J Respir Cell Mol Biol 1994, 11:644–645
29. Wong DTW, Weller PF, Galli SJ, Elovic A, Rand TH, Gallagher GT, Chiang T, Chou MY, Matossian K, McBride J: Human eosinophils express transforming growth factor-α. J Exp Med 1990, 172:673–681